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ENZYME REACTIONS IN APOLAR SOLVENTS. THE RESOLUTION OF BRANCHED AND UNBRANCHED 2-ALKANOLS BY PORCINE PANCREATIC LIPASE.

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Abstract: Straight-chain and branched 2-alkanols were subjected to enzyme catalyzed transesterification in organic solvent using porcine pancreatic lipase, high enantioselectivity being generally observed The method was applied to the synthesis of (R)-(-)-9-hydroxy-(E)-decenoic acid, a component of the queen bee mandibular gland pheromone

INTRODUCTION

The potential of enzymes as practical catalysts in organic syntheses is widely recognized [1] In particular lipases (triglycerol acyl-hydrolases EC 3 1 1 3) are powerful catalysts for the resolution of racemic alcohols The enantioselective acylation of hydrophobic alcohols in organic solvents by these insoluble enzymes makes the production of multigram quantities of optically enriched alcohols a facile laboratory procedure [2,3]

Porcine pancreatic lipase (PPL) is an inexpensive and stable enzyme that catalyzes enantioselective transesterification of a wide range of alcohols [2-4] Although commercial PPL also shows amylase and protease activity, it may be treated as an "off the shelf" transesterification reagent by organic chemists

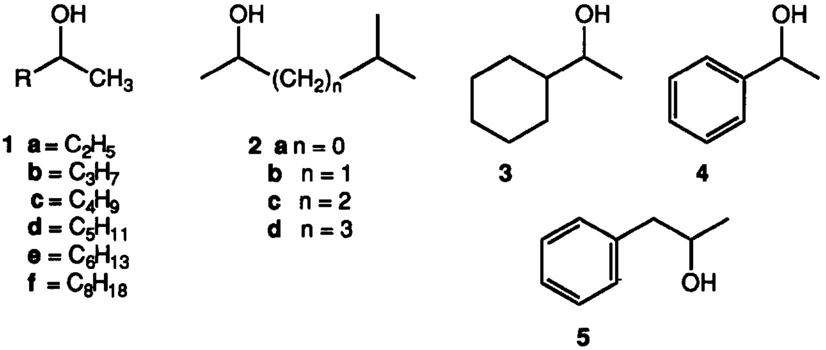
In the present study we have systematically investigated the effect of chain length and branching on the PPL catalyzed enantioselective transesterification of 2-alkanols, and describe a chemico-enzymatic synthesis of (R)-(-)- and (S)-(+)-9-hydroxy-(E)-decenoic acid, components of the queen bee mandibular gland pheromone complex [5]

Results and Discussion

Transesterification of racemic 2-alkanols, using PPL in ether and trifluoroethyl laurate or butyrate, was efficient at room temperature (Table 1) Reactions were stopped by filtration and the enantiomeric ratio (E) of the esterification reaction was determined from the degree of conversion and the enantiomeric excess (ee) of reactants and products [6,7] The initial rates,v_R, reported in Table 1 were determined using the pure R enantiomer (ee>0 98) of each alcohol under the conditions employed for resolution of the racemic substrate

The enantiomeric ratio (E) is independent of substrate and enzyme concentration as well as the extent of conversion as long as the reaction is irreversible [7b] While reversibility can become a significant problem at conversions >40%, enantiomeric ratios (E) were calculated assuming irreversible processes High enantioselectivity is most efficiently achieved under conditions of rapid trans-esterification and irreversible conditions The catalytic process involves initial acylation of PPL by the transesterification agent This is followed by enantioselective acylation of the secondary alcohol substrate by the acyl-enzyme complex Use of triflouroethyl laurate or butryate speeds acyl enzyme formation and releases the weakly nucleophilic trifluoroethanol which does not compete with the substrate alcohol in enzyme acylation [3d] Enantiomeric ratios (E) in the range below 100 are good measures of the efficiency of these resolutions However, no attempt was made to distinguish between values greater than 100 as E becomes increasingly sensitive to very small errors in measurement of ee

SCHEME I



1 a = C_2H_5
b = C_3H_7
c = C_4H_9
d = C_5H_{11}
e = C_6H_{13}
f = C_8H_{18}

2 a n = 0
b n = 1
c n = 2
d n = 3

3

4

5

In the series of linear 2-alkanols studied (**1a-f**) there is a rapid increase in the degree of enantioselectivity as the alkyl group is changed from C_2H_5 to C_4H_9 (**1a-c**) Further chain extension has little effect on the degree of enantioselectivity (cf , **1d-f**). Although the alcohol with the largest alkyl chain in our study was decanol (**1f**), both 2-dodecanol and 2-hexadecanol have been resolved under similar conditions using PPL [3b] The present work suggests that the minimum requirement for enantioselectivity in 2-alkanol transesterifications is a propyl or isopropyl substituent Substitution of the C3-H in 2-butanol (**1a**) with a methyl to give 3-methyl-2-butanol (**2a**) results in a sixteen fold increase in enantioselectivity Addition of this methyl branch has an effect comparable to extending a linear chain by a single methylene (eg , compare 2-butanol, **1a**, with 2-pentanol, **1b**). Enantioselectivity is only weakly influenced by the presence of branch methyls in alcohols (**2b-d**) that possess longer alkyl chains. The observation that 1-cyclohexylethanol (**3**) exhibits an enantiomeric ratio only slightly higher than 2-pentanol (**1b**) suggests that the flexibility of appended alkyl chains rather than their absolute size is the major determinant of enantioselectivity The situation is less clear for phenyl substitution While there is an increase in enantioselectivity in the series 2-butanol (**1a**) < 3-methyl-2-butanol (**2a**) < 1-cyclohexylethanol (**3**) < 2-octanol (**1e**) < *sec*-phenethyl alcohol (**4**), replacement of the phenyl group in **4** by a benzyl group (**5**) results in a sharp drop in enantioselectivity

Several factors affect the rate of PPL catalyzed reactions in organic solvents Dehydration of the lipase increases its stability and stereoselectivity,^{8a,d} while catalytic activity is decreased ^{8c} Comparison of initial rates of **1e**, **2c**, **2d** with their overall conversions indicate that, although **2c** and **2d** had higher initial rates, their actual rate decreased more over time than the rate of **1e** We made no attempt to determine K_m or V_m for the secondary alcohols used in this study It is possible that the observed reversal of relative rates over time is due to differences in binding, the branched substrates **2c** and **2d** being more strongly bound to the enzyme but having a lower maximum velocity than the linear alcohol **1e**

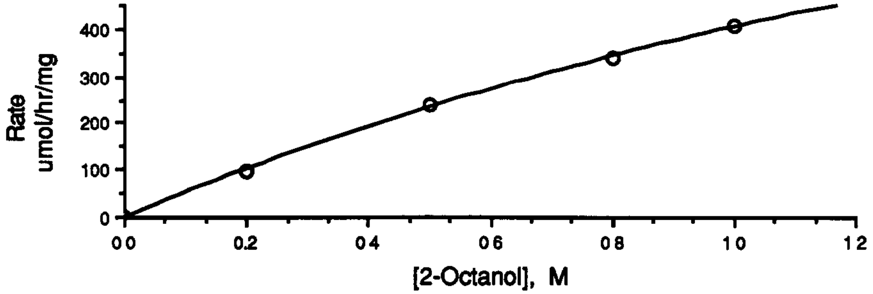
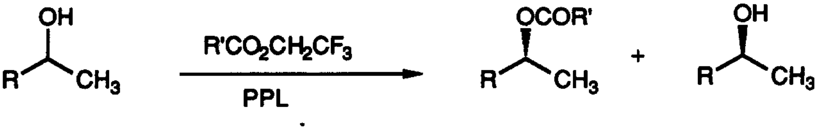


Fig 1 Initial rates of the transesterification reaction between (1)-2-octanol and trifluoroethyl laurate (1 25M) in ether catalyzed by porcine pancreatic lipase (PPL)

Various ways were considered for increasing the rate of transesterification. In ether at high (1)-2-octanol concentration (0 2-1 0 M) and acyl donor concentrations (1 25 M trifluoroethyl laurate) the initial rate increased in an almost linear fashion with increasing 2-octanol concentration (Fig 1) Under these conditions lipase concentrations of 0.33 g/mL or higher can be kept in solution using a magnetic stirrer Compared to the conditions used in Table 1, the use of higher substrate and lipase concentrations may result in a five-fold increase in the rate of 2-octanol transesterification Shaft-driven stirrers, or shakers or mixers designed for handling slurries could further increase lipase loads and lead to further rate increases

Table 1. Resolution of Linear and Branched 2-Alkanols



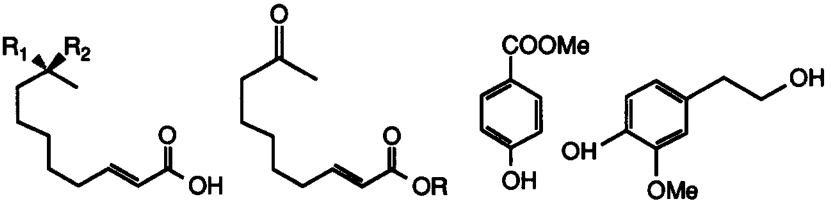
Structure	Time h	ees	eep	conversion	E	Initial Rate nMmg/hr
1a	23 0	0 216	0.342	0 387	2 5	
1b	19 5	0 428	0 943	0 312	52	
1c	19 5	0 603	0 968	0 384	>100	
1d	19 5	0 696	0 955	0 421	92	
1e	19 5	0 932	0 927	0 501	90	190 4
1f	23 0	0 383	0 977	0 282	>100	
2a	24 0	0 199	0 942	0 174	41	
2b	41 5	0 382	0 982	0 280	>100	
2c	41.5	0 942	0 977	0 491	>100	218 0
2d	41 7	0 865	0 933	0 482	80	220 9
3	31 0	0 350	0 956	0 268	62 [a]	
	89 25	0 398	0 960	0 293	71 [a,b]	
4	31 0	0 383	> 0 97	n/d	>100 [a]	
	89 25	0.658	0 967	0 405	>100 [a,b]	
5	43 0	0 230	0 894	0 205	22	
	30 5	0 112	0 929	0 108	29 [a]	
	89 25	0 270	0 898	0 231	25 [a,b]	

All runs were carried out in Et_2O (5 mL), alcohol (0 5 M), trifluoroethyl laurate (1 0 M), and PPL (1 0 g), except where noted [a] Alcohol (0 4 M), trifluoroethyl butyrate (1 2 M) and PPL (1 0 g) [b] Reaction run in hexanes (5 mL)

Chemicoenzymatic Route to 9-Hydroxy-(E)-decenoic Acid

To determine whether functional groups in remote positions have a detrimental effect on enantioselectivity similar to that observed for introduction of the benzyl group in **5**, we examined the resolution of unsaturated acids and esters that are precursors of 9-hydroxy-(E)-decenoic acid (9-HDA, **6**) This hydroxy acid is a major component of the queen bee mandibular gland pheromone, which is responsible for retinue formation in the honey bee, *Apis mellifera* L[5,11-12] The other components of the pheromone blend are, 9-oxo-(E)-decenoic acid (9-ODA) (**7**), the major component, and smaller amounts of methyl p-hydroxybenzoate (HOB) (**8**) and homovanillyl alcohol (HVA) (**9**)[13] Although the optical activity of queen bee produced 9-HDA is variable (ee, 70-95%), it is the R-(-) enantiomer (**6a**) that predominates and can maintain queenless swarms[13,14]

SCHEME II



6 **a** R_1=H, R_2=OH
bR_1=OH R_2=H

7

8

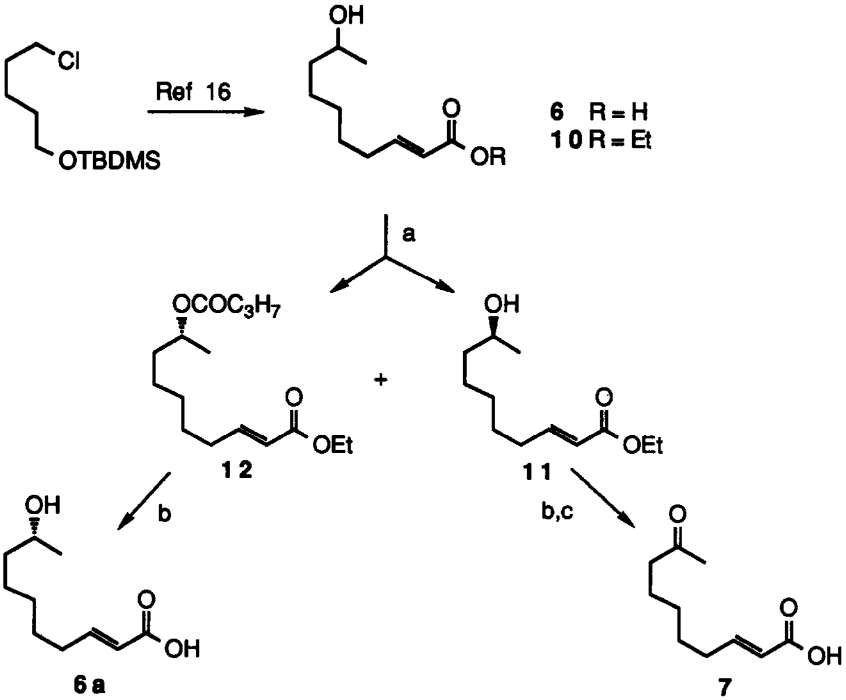
9

Racemic 9-HDA is readily prepared by reduction of 9-ODA, for which a number of syntheses have been published.[16] Since **6** is a 2-alkanol we considered it likely that it would be efficiently resolved by PPL transesterification Furthermore, the unwanted S-(+) enantiomer may be oxidized to 9-ODA (**7**) and used in the formulation of synthetic mandibular gland pheromone complex, conserving expensive material

Efficient (E> 100) kinetic resolution of hydroxyalkanoates has been described for the lipase catalyzed (*Pseudomonas sp* K-10) cleavage of t-butyl-7-acetoxyoctanoate in aqueous solution,[17a] and for the enzymatic lactonization of 7-hydroxyoctanoic acid in anhydrous isooctane using *Pseudomonas sp* lipase (AK, Amano)[17b] Racemic 9-hydroxydecenoic acid (**6**) was prepared by the method of Kandil and Slessor[18] Kinetic resolution of **6** by PPL under transesterification conditions using trifluoroethyl laurate as the acylating agent was negligible The ee of unreacted **6** after 25 h was only 0 064[19] Under similar conditions, the corresponding ester, ethyl 9-hydroxydecenoate (**10**), was efficiently resolved (E=60-70) using either trifluoroethyl laurate or butyrate as acylating agent Although reaction with the latter was somewhat slower (Table 2) its volatility facilitated separation from other reaction products and led us to select it as the acylating agent for preparative scale resolutions When butyric anhydride was used as the acylating agent poor selectivity was observed[20] Termination of reaction at 52% conversion yielded (S)-ethyl-9-hydroxydecenoate (**11**) (45%, ee=96%), and (R)-ethyl-9-butyroxydecenoate (**12**, 46%, ee=89%)

Saponification of **12** gave (R)-(-)-9-hydroxy-decenoic acid (**6a**, 84%) while saponification of **11** yielded (S)-(+)-9-hydroxydecenoic acid (**6b**, 80%)

SCHEME III [a]



[a] (a) PPL, $C_3H_7CO_2CH_2CF_3$, Et_2O, 25-68 h, (b) 3M NaOH, H_2O, i-PrOH, 24 h, (c) Jones reagent, acetone, 0 0C

Bulk and flexibility of alkyl residues attached to the chiral carbon as well as the position and nature of other functional groups and chain length are important factors in determining the enantioselectivity of PPL mediated transesterification of 2-alkanols With the exception of 2-butanol (**1a**) and 1-phenyl-2-propanol (**5**) all linear and branched chain 2-alkanols examined were readily resolved Substitution of a cyclohexyl or phenyl group adjacent to the hydroxyl bearing carbon increases enantioselectivity, whereas an adjacent benzyl group leads to a marked decrease in selectivity With remote functional groups the effect on enantioselectivity varies Shear bulk in the 3 position of 2-alkanols is an important factor in determining enantioselectivity Alcohols containing remote saturated and unsaturated esters exhibit lower enantioselectivities compared to those with saturated hydrocarbon chains No enantioselectivity was observed when alkanols contained remote terminal carboxylic acids

Table 2. PPL Catalyzed Resolution of ω-Hydroxyalkanoates.

Run	R	Acylating Agent	Time h	ee$_s$	ee$_p$	%	E
1.[a]	$-(CH_2)_5CO_2Et$	TFEL	25	0.515	0.954	35.1	70
2.[a]	"	TFEB	46	0.728	0.943	43.6	75
3.[b]	$-(CH_2)_5CH=CHCO_2H$	TFEL	25	0.064	n/d	n/d	n/d
4.[a]	$-(CH_2)_5CH=CHCO_2Et$	TFEL	25.3	0.420	0 961	30.4	75
5.[a]	"	TFEB	46	0.474	0 950	33.3	62
6[c]	"	Butyric Anhydride	145	0.607	0 677	47.3	10

[a] Alcohol (0.5 M), Trifluororethyl ester (1.0 M), PPL (1.0 g) Et_2O (5 mL). [b] Et_2O (3 mL), PPL (0.6 g).
[c] Alcohol (0.9 M), Butyric anhydride (1 2 M), PPL (6.5 g), Et_2O (50 mL)

EXPERIMENTAL SECTION

GENERAL METHODS. Porcine Pancreatic Lipase (Type II) was obtained from Sigma Chemical Company and was used as received The listed activity was 16 units per mg of solid using olive oil at pH 7 7 and an incubation time of 30 min. With the following exceptions the 2-alkanols were commercially available and were used as received 2-Heptanol (**1d**), 3-methyl-2-butanol (**7**), 4-methyl-2-pentanol (**2b**), and 5-methyl-2-hexanol (**2c**) were prepared by reduction (LAH or $NaBH_4$) of the corresponding commercially available ketones 2-Decanol (**1f**) was prepared by oxymercuration of 1-decene[21] and 6-methyl-2-heptanol (**2d**) was prepared by catalytic reduction of commercially available 6-methyl-5-hepten-2-ol 2,2,2-Trifluroethyl laurate (bp$_{0\ 5}$ 106-1080C) and 2,2,2-trifluoroethyl butyrate (bp 109-1110C) were prepared from the corresponding acid chloride and trifluoroethanol. Reagent grade anhydrous ether was used as received for resolution experiments.

Column chromatography was performed on silica Gel (Merck Kieselgel 60, 230-400 mesh). Gas chromatography was performed on a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector and employing a J/W fused silica DB-1 capillary glass column (15 m x 0.25 mm). Reactions were monitored using the following temperature program: initial temperature, 600C for 1 min, rate, 20 deg/min, final temperature, 2500C for 2 min The enantiomeric excess of optically enriched alcohols was determined by derivatization with acetyl (S)-lactyl chloride,[14] and GC analysis of the resultant diastereoisomeric mixture used the following run program initial temperature, 700C; rate 5 deg/min; final temperature 2500C for 5 min ^1H NMR (400 MHz) and ^{13}C

NMR (100 6 MHz) were recorded on a Bruker WM 400 spectrometer and 100 MHz ^{1}H NMR on a Bruker WP100SY in $CDCl_3$ with $CHCl_3$ (δ 7 25) as internal standard Chemical Ionization (GC/MS) spectra were obtained on a Hewlett-Packard 5985B GC/MS system with isobutane as the ionizing gas Elemental analyses were performed by Mr M Yang (Department of Biological Sciences, SFU) on a Perkin-Elmer Model 240 elemental analyzer IR spectra were run on a Perkin-Elmer 1310 or 599B spectrophotometer as neat films on NaCl plates Optical rotations were measured on a Dr Kernchen Digital Automatic Saccharimeter

Lipase Mediated Resolution of 2-Alkanols. General Procedure. A mixture of the 2-alkanol (2 6 mmol), trifluoroethyl laurate or trifluoroethyl butyrate (5 0 mmol) and porcine pancreatic lipase (Sigma Type II) (1 0 g) in Et_2O (5 mL) was stirred at room temp Reactions were stopped (19-89 h) by filtration through a bed of Celite to remove the enzyme and the solvent was removed *in vacuo* The enantiomeric excesses of unreacted substrate alcohols were determined by treatment of a portion of the crude reaction concentrate with acetyl (S)-lactyl chloride followed by GC analysis Column chromatography (10% EtOAc/hexanes) or, in the case of the more volatile alcohols, exposure of the crude reaction to high vacuum, yielded the acylated substrate, contaminated with trifluoroethyl laurate in some cases Reduction of a sample of the acylated substrate with LAH in Et_2O, followed by derivatization with acetyl (S)-lactyl chloride and GC analysis gave the enantiomeric excess of the acylated product In the case of the hydroxy acids/esters (**6**,**10**), the laurate or butyrate ester was removed by hydrolysis and the (S)-lactate ester was prepared after treatment with diazomethane On the column used for GC analysis the (S)-lactate ester of (R)-sulcatol eluted prior to the (S)-lactate ester of (S)-sulcatol[8a] Likewise the (S)-lactate ester of (R)-**10** eluted prior to that of the (S)-lactate ester of (S)-**10**.[18] The configurations of the diastereoisomeric lactates prepared in this study were assigned by determination of their relative retention times under conditions identical to those used for the separation of the (S)-lactate esters of sulcatol and **10** It was assumed by analogy with the foregoing examples that the (S)-lactate esters of the (R) enantiomers eluted prior to the (S)-lactate esters of the (S) enantiomers If this assumption holds, then in all cases it was the R enantiomer which was preferentially acylated enzymatically The laurate and butyrate ester products were not characterized After removal of the acyl group the optically enriched alcohols showed GC retention times (for both the alcohol and the (S)-lactate derivative) which were identical to the racemic starting material.

Kinetic Resolution of Ethyl 9-hydroxydecenoate (10). Ethyl 9-hydroxydecenoate (**10**) (4 28 g, 20 0 mmol, 0 27 M), trifluoroethyl butyrate (8 05 g, 47 3 mmol, 0 63 M) and PPL (10 0 g) were stirred in anhydrous Et_2O (75 mL) for 67 5 h The enzyme was removed by filtration through Celite and the filtrate was washed with saturated sodium bicarbonate (50 mL), water (50 mL) and satd aqueous sodium chloride soln (50 mL) After drying ($MgSO_4$) the soln was filtered and the solvent removed *in vacuo* The crude product was chromatographed on silica Gel (110 g) eluting with 5% (400 mL) and 7% (400 mL) EtOAc/hexanes Combination of relevant fractions yielded (R)-ethyl-9-butyroxydecenoate (**12**) (2 51 g, 46%) Elution with 10% (400 mL), 30% (200 mL) and 50% (400 mL) EtOAc/hexanes and combination of the relevant fractions yielded (S)-ethyl-9-hydroxydecenoate (**11**) (1 94 g, 45%) with an ee of 0 96

(S)-(+)-9-Hydroxydecenoic acid (6b). Hydroxyester **11** (1.94 g, 9.1 mmol) was stirred for 22 h with a solution of i-PrOH (5 mL) and 2 M NaOH (30 mL) The reaction mixture was extracted with CH_2Cl_2 (2 x 50 mL) and the aqueous layer was then acidified with 6 M HCl (15 mL) and extracted with Et_2O (150 mL) The organic layer was washed with satd aqueous sodium chloride soln. (50 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude product was distilled under vacuum in a Kugelrohr oven to yield a colorless liquid (1 36 g, 80%) $[\alpha]^{21\ 6}_{D}$ = +8 51 (c= 12 92, MeOH) (ref.[18] $[\alpha]^{23}_{D}$ = +5.2 (c = 7.62, EtOH)), IR (film) 3100-3500, 2990, 2950, 2880, 2500-2800, 1710, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 400MHz) δ 1 18 (d, 3H, J=6.4 Hz), 1 30-1.52 (m, 9H), 2 23 (q, 2H), 3 80 (m, 1H), 5 81 (d, 1H, $J_{2,3}$=15 6 Hz), 6 50 (bs, 1H), 7 05 (dt, 1H, $J_{2,3}$=15 6 Hz, $J_{3,4}$=6 4 Hz); ^{13}C NMR ($CDCl_3$, 100 6MHz) δ 171 20, 151 66, 68 02, 38 89, 32 05, 28 98, 27.73, 25 31, 23 24 Anal Calcd for $C_{10}H_{18}O_3$· C, 64.49, H, 9 74 Found C, 64 35; H, 10 03.

(R)-(-)-9-Hydroxydecenoic acid (6a). The butyroxy ester **12** (2 51 g, 9 3 mmol) was saponified in a solution of iPrOH (5 mL) and 3 M NaOH (20 mL) for 24 h. The crude product was isolated using the same work-up as above and the crude product was distilled *in vacuo* in a Kugelrohr oven to yield a colorless liquid (1 46 g, 84%) The enantiomeric excess of the corresponding methyl ester was determined (ee=0 894) and the enantiomeric rate ratio (E) of the transesterification was calculated to be 70 $[\alpha]^{20\ 5}_{D}$ = -7.95 (c = 16 48, MeOH) (ref.[18] $[\alpha]^{23}_{D}$= -5 4 (c = 21 0, EtOH)), IR (film) 3100-3500, 2960, 2920, 2850, 1690, 1650 cm-1, ^1H NMR ($CDCl_3$, 400MHz) δ 1.16 (d, 3H, J=6 4 Hz), 1 25-1 50 (m, 9H), 2 24 (q, 2H), 3 80 (m, 1H), 5.79 (dd, 1H, $J_{2,3}$=15.6 Hz, $J_{2,4}$=0 8 Hz), 7 02 (dt and bs, 2H, $J_{2,3}$=15 6 Hz, $J_{3,4}$=6 4 Hz), ^{13}C NMR ($CDCl_3$, 100 6MHz) δ 171 41, 151 74, 120.72, 68 02, 38 93, 32 07, 28 99, 27 73, 25 34, 23 31 Anal. Calcd for $C_{10}H_{18}O_3$ C, 64 49, H, 9.74 Found C, 64 34, H, 10 01

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6 The enantiomeric excess of the unreacted starting materials and the products (after reductive deacylation) was determined by derivatization with acetyl (S)-lactyl chloride, followed by GLC analysis See ref 14

7 The enantiomeric ratio (E) is a measure of the enzyme discrimination between two competing enantiomers, and is the ratio of the rate constants for the fast and slow enantiomers

The enantiomeric ratio (E value) was calculated from

$$E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)] = \ln[1-c(1+ee_P)]/\ln[1-c(1-ee_P)]$$

where $c = ee_S/ee_S+ee_P$ The listed E values are averages calculated from both ee_S and ee_P See: (a) Chen, C -S , Fujimoto, Y , Girdaukas, G ; Sih, C J *J Am Chem Soc* **1982**, *104*, 7294-7299. (b) Chen, C -S , Wu, S -H , Girdaukas, G , Sih, C J *J. Am Chem Soc* **1987**, *109*, 2812-2817.

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19 Poor selectivity has also been reported for the lipase catalyzed hydrolysis of acyloxyalkanoic acids using *Pseudomonas sp* (Amano AK and K-10) lipases See ref 17a

20 Poor selectivity was also observed in the acylation of long chain secondary alcohols with anhydrides, using Lipase Amano P supported on Celite See ref 3a

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